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**14. ABSTRACT**

Heretofore, efforts to develop implantable chemical sensors for real-time clinical monitoring of glucose subcutaneously (SQ) in diabetic patients have been stymied by the unreliable analytical results owing largely to biocompatibility problems induced by sensor implantation (e.g., inflammatory/foreign body response). The goal of this research program is to explore and optimize the chemistries required to fabricate implantable amperometric glucose sensors with outer polymeric coatings that slowly generate low levels of nitric oxide (NO). The local generation of NO has been shown to enhance the biocompatibility of the implanted sensors by decreasing the inflammatory response. The focus of this research has been to develop new polymeric coatings (biomedical hydrogels and polyurethanes) that possess immobilized copper ion sites that will serve as catalytic surfaces for in situ conversion of endogenous nitrosothiol species (RSNO) (e.g., nitrosoglutathione, nitrosocysteine, etc.) to NO, thereby providing a local sustained generation of NO species at the surface of the implanted sensors. Preliminary experiments indicate RSNO levels within the SQ fluid of rats are sufficiently generate local NO to reduce the inflammatory response at the implantation site, using test devices coated with polymers containing immobilized copper ion sites. Finally, functional needle type SQ glucose sensors were prepared to demonstrate appropriate analytical performance for glucose measurements. These sensors will provide the basis of assessing if NO generation chemistries are compatible with glucose sensing chemistry?

**15. SUBJECT TERMS**
Biocompatibility; sensors; inflammation; angiogenesis; polymeric coatings; nitric oxide

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## INTRODUCTION

To date, efforts to develop implantable chemical sensors for real-time clinical monitoring of glucose subcutaneously (SQ) in diabetic patients have been stymied by the unreliable analytical results owing largely to biocompatibility problems induced by sensor implantation (e.g., inflammatory/foreign body response). The goal of this research program is to explore and optimize the chemistries required to fabricate implantable amperometric glucose sensors with outer polymeric films that slowly generate low levels of nitric oxide (NO) from endogenous nitrosothiol (RSNO) species likely to be present in interstitial fluid. The local generation of NO is expected to greatly enhance biocompatibility of the implanted sensors by decreasing the inflammatory response and promoting angiogenesis as well as wound healing at the implant site.

Although recent studies [1] with thin NO release polymer coatings on SQ glucose sensors have already demonstrated that local NO release can reduce initial inflammatory response of surrounding SQ tissue, the reservoir of NO precursors that can be retained in such thin polymeric coatings is too low to achieve prolonged NO release (>1 d) at physiologically relevant levels. Hence, for long-term sensor implants (weeks to months), a completely new strategy to locally generate NO at the surface of the devices will be required. The focus of this research program is to develop new polymeric coatings (biomedical hydrogels and polyurethanes) that possess immobilized copper(II) ions or other sites (e.g., organoselenium) that will serve as catalytic surfaces for *in situ* conversion of any endogenous nitrosothiol species (e.g., nitrosoglutathione, nitrosocysteine, etc.) to NO, thereby providing a sustained local generation of NO species at the surface of the implanted sensors. Experiments will be undertaken to assess the relative variations in the levels of RSNO substrates within the SQ fluid of rats, by implanting test devices in SQ region of rats that were coated with polymeric materials containing a Cu(II)-complex material. Finally, functional needle type SQ glucose sensors will be prepared that possess appropriate analytical performance for glucose measurements. These functional sensors will be used as the basis to ensure that the NO generating chemistry is compatible with the sensing chemistry and that its function will not be compromised by the use of these novel biocompatible coatings.

## BODY

Significant progress has been made over the past year toward all of the goals in the approved Statement of Work for this project which are aimed at developing and testing a completely new approach to enhance the biocompatibility and concomitant analytical performance of glucose sensors designed for continuous subcutaneous measurements of blood glucose levels. The three main project aims are: 1) synthesis and characterization of new polymers (derivatized polyurethanes and poly(hydroxyethylmethacrylates)) containing immobilized copper(II) ion sites (via Cu(II)-cyclen complexes) and potentially other catalysts that can generate NO from RSNO species as a next generation of anti-inflammatory coatings; 2) *in vivo* testing of NO generation from subcutaneous tissue with sham sensor devices coated with polymers containing a Cu(II)-cyclen complex to mediate the inflammatory response around implants, and 3) fabrication of functional needle-type glucose sensors in our laboratory with the new NO generation coatings, and demonstrating that NO generation chemistry does not decrease the analytical functionality of such glucose sensing devices.

1) <u>Synthesis and Characterization of New Polymers that Catalytically Generate NO:</u> A new material was synthesized and characterized that contains a modified Cu(II)-cyclen compound covalently attached to a cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA) and it was demonstrated that this new material is able to catalytically generate NO from RSNO species at physiological pH values [2]. Because pHEMA is a high water-uptake polymer, NO generation from small, diffusible RSNO species present in blood and subcutaneous fluid can diffuse into the bulk of the polymer and potentially generate NO within the polymer (previous work in our laboratory showed NO generation in hydrophobic polymers took place only at the polymer/fluid interface). Additionally, because Cu(II)-cyclen complex is covalently attached to the polymer backbone, leaching of the copper(II) complex *in vivo* surrounding the implant site should not be a significant issue.

In addition to immobilized Cu(II) catalytic sites, an alternate class of polymeric materials have been discovered recently in this laboratory (also part of an ongoing NIH grant to develop NO generation coatings for vascular graft applications) that incorporate organoselenium compounds immobilized on polymers. The organoselenium species are able to catalytically generate NO from RSNO species by mimicking the selenium-containing enzyme, glutathione peroxidase (GPx) [3]. This is an attractive alternative to using copper-based chemistry because of the toxicity concerns associated with introducing copper species to physiological systems. Indeed, we have found that the newly discovered organoselenium catalysts are robust catalysts which can be used to prepare electrochemical RSNO sensors that function reliably for measuring RSNO species in blood [4].

2) <u>*In Vivo* Testing of NO Generation vs. NO Release Within Subcutaneous Rat Tissue</u>: Initial *in vivo* testing of sham devices coated with a Cu(II)-cyclen complex [5] blended into Pursil (a biomedical grade polyurethane) was carried out concurrently with the development of the covalently attached materials described above. Implanted devices with dimensions 1.5 cm in length x 1 mm width were fabricated and coated with 3 different test polymeric materials, a positive control polymer that releases NO polymer using a lipophilic diazeniumdiolate species we have previously described [6]; a negative control coating that neither releases nor generates NO; and a test material coated with the PU polymer containing 2 wt% of Cu(II)-cyclen compound that catalytically generates NO from RSNO species. Devices were implanted in the subcutaneous region of rats for 7 days and then the surrounding tissue was explanted and examined for inflammatory marker cells by cell staining. In total, 16 animals were used to test a total of 48 coated devices (each animal received 3 implants, one positive control, one negative control, and one test material). Figure 1 illustrates the level of inflammatory response measured for the 3 different groups of SQ implanted sham devices. Data were normalized to the degree of inflammatory cell response found with the positive control in each animal and then compiled.

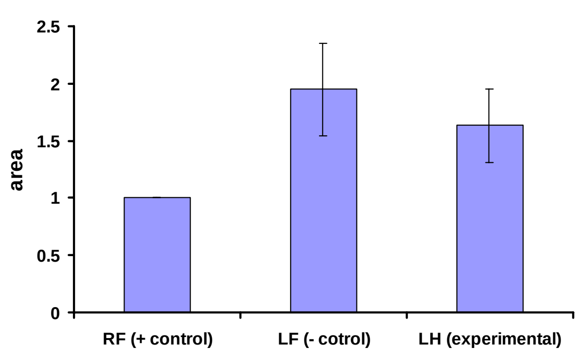


**Figure 1:** Illustrates the reduction in the inflammatory response toward devices that release NO (+ control) or generate NO (experimental) compared to plain polymer coatings (-control). The area plotted on the y-axis is the ratio of the surface area of inflammatory cells/total cells.observed (n=10 animals). Results were standardized to the +-control within each animal.

As can be seen in Fig. 1, there is clearly a pattern of reduced inflammatory response for the NO release (positive control) and NO-generating (test) materials compared to the negative control device that does not release or generate NO. It should be noted that in the process of developing the surgical procedure used to

implant the devices and device fabrication there were certain problems in the initial experiments (e.g., sham devices slid into different locations under the skin as the animals were freely moving, etc.); hence, data from only 10 animals are included in this initial comparative study. Nonetheless, this first set of *in vivo* results reconfirms that NO release polymers significantly decreases inflammatory response (as we found previously using functional NO release glucose sensors in rats [1]) in the subcutaneous region, and that the Cu(II)-ligand based polymers also appear to provide some marginal improvement in decreasing inflammatory response. A key question is whether this decrease observed for the Cu(II)-ligand polymer coatings is truly due to RSNO decomposition in the SQ region. Thus, during the next period of this project, in addition to testing new NO generating materials in a similar manner, we will also prepare needle type versions of our electrochemical RSNO sensor [4], in an effort to confirm the presence and relative concentrations of RSNO species in the SQ fluid of rats.

3) Fabrication of Functional Needle-type Glucose Sensors: A new graduate student (Ms. Qinyi Yan) was recently recruited to begin developing functioning needle type glucose sensors. Ultimately, she will test their compatibility with various NO generating chemistries (both the copper(II) and organoselenium based systems). The enzyme based needle-type glucose sensors have been fabricated as described by Bindra, et. al. [7]. Briefly, a 10 cm piece of 0.008 in. dia. Teflon coated platinum/iridium (Pt/Ir) wire is used as the body of the sensor. A small window of the Teflon coating was removed and the exposed Pt/Ir wire served as the working electrode for the amperometric device. A silver/silver chloride reference electrode was then wrapped around the Pt/Ir wire. An enzyme layer containing glucose oxidase was then deposited on the working electrode. A protective outer layer of hydrophilic Tecoflex polyurethane was then applied.

As shown in Figure 2, such needle type sensors respond linearly to glucose concentrations in the range of 0 to 30 mM. Further, the response times toward changes in glucose are rather rapid (< 30 sec). Our next objective is to study the effect of using different outer polymer coatings that possess the immobilized Cu(II) and RSe sites for generating NO from RSNO species. For example, use of the same Tecoflex PU outer coating but with immobilized Cu(II)-cyclen complex attached directly to the polymer or further coated with a thin layer of the new Cu(II)-cyclen polymethacrylate hydrogel will be the first systems to be examined. In addition, we will attempt to immobilize our newest organoselenium catalysts [3] on the surface of the polyurethane coating. In these upcoming studies, we will compare the analytical response and performance of these needle type glucose sensors with and without the NO generating chemistry present.

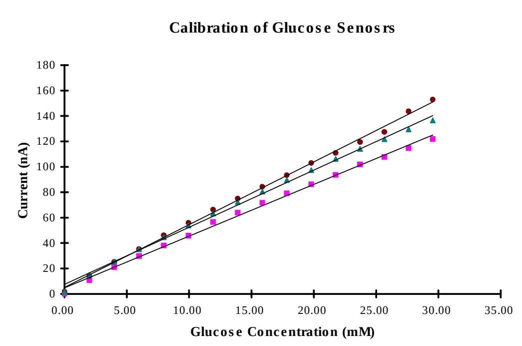


**Figure 2:** Calibration curves for needle-type glucose sensors constructed demonstrating linear response toward increasing glucose concentration

## KEY RESEARCH ACCOMPLISHMENTS (during year 1):

- Synthesized Cu(II)-cyclen compound covalently attached to a cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA) and it was demonstrated that this new material is able to catalyically generate NO from RSNO species at physiological pH values.

- Synthesized new organoselenium compounds immobilized on cellulose filter paper and polyethylenimine that are able to catalytically generate NO from RSNO species by mimicking the selenium-containing enzyme, glutathione peroxidase (GPx).

- Demonstrated in initial *in vivo* studies that NO-generating polymer coatings based on a lipophilic Cu(II)-cyclen complex doped within a medical grade polyurethane material modestly reduces inflammatory response in tissue surrounding subcutaneous implanted sham devices in rats for a 7 day period.

- Fabricated enzyme-based needle-type glucose sensors that exhibit linear response to glucose in the concentration range of 0-30 mM.

**REPORTABLE OUTCOMES:**

Conference presentations:

-Sangyeul Hwang, Wansik Cha, Mustafa Musameh, Hyoung-Sik Yim, Mark E. Meyerhoff "Nitric oxide generating materials based on immobilized catalysts for nitrosothiol decomposition in blood: a novel approach for creating thromboresistive polymers," Methods in Bioengineering Symposium, Cambridge, MA July 18, 2006.

-Mark E. Meyerhoff, "Electrochemical Sensors in Medicine: Meeting Needs for the 21$^{st}$ Century," SEAC Awards Symposium, PittCon 2006, Orlando. FL, March 20, 2006.

-Mark E. Meyerhoff, "Electrochemical Sensors for Nitrosothiols Using Immobilized Chemical and Biochemical Catalysts," American Chemical Society National Meeting, Analytical Electrochemistry Awards Symposium, San Franscisco, CA, September 12, 2006.

Publications:

-M. Frost and M. E. Meyerhoff, "*In Vivo* Chemical Sensors: Tackling Biocompatibility," Anal. Chem., in press, 2006.

**CONCLUSIONS**

During the past year, a new material was synthesized and characterized that utilizes Cu(II)-cyclen compound covalently attached to a cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA). This material generates NO from RSNOs species present in solution at physiological pH. Initial *in vivo* evaluation of this material will begin shortly, as a coating on an implanted SQ sham device. Further, preliminary *in vivo* studies using a lipophilic Cu(II)-cyclen compound blended into a biomedical grade polyurethane have demonstrated that an apparent reduction in inflammatory response surrounding subcutaneous devices implanted in rats. This supports the notion that sufficient RSNO species exist in interstitial fluid in the subcutaneous region of rats to generate NO to help mediate the inflammatory response. Direct proof that NO is the agent causing the observed improvement still needs to be obtained. This will be accomplished by two different approaches, either the implantation of the novel needle type RSNO sensor in the subcutaneous region of live rats (as described in the initial grant application) and/or by using the organoselenium material developed as an alternate approach for catalytic generation of NO. If implanted devices coated with the new organoselenium materials also exhibit the same decrease in inflammatory response as the devices coated with the Cu(II)-containing materials, then it is highly probable that NO is the species responsible for the reduced inflammatory response. We have also been able to fabricate a functional enzyme-based needle-type glucose sensor which

will allow work to begin on testing the compatibility of the new NO-generating coatings with the glucose sensing chemistry, an important fundamental goal of this Army project.

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